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SYNCHRONIZED RHYTHMIC CONTRACTIONS AMONG FIVE GONADAL LOBES IN THE SHEDDING SEA URCHINS: COORDINATIVE FUNCTION OF THE ABORAL NERVE RING

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Abstract

The gonadal lobe of the spontaneously shedding sea urchin, *Temnopleurus toreumaticus,* contracts rhythmically. The gonadal movement following electrical or KCl stimulation is a summed response of the rhythmic and arrhythmic contractions. The lobes pretreated with $MgCl_2$ show only the latter type. Contraction induced by acetylcholine is the latter type. The aboral nerve ring (ANR) connects proximal ends of five gonoducts. In a preparation with intact ANR, the rhythmic contraction occurs synchronously in all five gonadal lobes. When the ANR is separated into two sectors, lobes belonging to the same sector contract synchronously, but those belonging to the different sectors do not.

These results demonstrate rhythm generators (pacemakers) in the ANR that are responsible for individual and synchronous contractions among the gonadal lobes.

Introduction

Eggs and sperm of sea urchins are used widely in various fields of modern biology. These gametes are routinely obtained by injecting isotonic KCl solution (Palmer, 1937; Harvey, 1939) or acetylcholine (ACh) dissolved in sea water (Iwata and Fukase, 1964a) into the test cavity, or by stimulating the animal electrically (Iwata, 1950, 1976; Harvey, 1952). Iwata and Fukase (1964b) speculated that both KCl and electrical stimulation directly affect the gonadal muscle (Palmer, 1937; Kawaguti, 1965), while ACh stimulates its cholinergic receptors.

Iwata (1976) reported that an increase in the internal pressure of gonads, possibly due to muscular contraction, results in spontaneously or artificially induced shedding.

Therefore, we investigated the shedding process by simultaneously recording gonadal contractions in five gonadal lobes after surgery on putative neural pathways. The results clearly show that the rhythmicity in the contraction pattern is synchronized among lobes and that the synchronization is achieved by coordination of neural activities in the aboral nerve ring (ANR).

Material and Methods

Sea urchins [*Temnopleurus toreumaticus* (Leske)], 3 to 5 cm in test diameter, were collected in the Seto Inland Sea near the Ushimado Marine Laboratory of Okayama University. Experiments were carried out mostly during the breeding season from July to August. No sexual differences were found in the response pattern.

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Abbreviations: ACh, acetylcholine; ANR, aboral nerve ring.

The animals were cut along the equator of the test and the gonad bearing aboral halves (vol. 3 to 7 ml) was used. All spines were scraped off with rough whetstone to avoid any mechanical disturbance. Digestive canals were cut with a razor blade.

A 2.5 cm diameter hole was made in the center of a horizontal shelf found on the upper portion of an acrylic experimental vessel (10 × 10 × 10 cm). The specimen was placed aboral side down on the hole and the cup-shaped test was filled to the upper edge of the gonads with sea water.

A straw placed vertically on the central surface of the gonadal lobe was connected to a high-gain force displacement transducer (Nihon Kohden, SB-1T-H) and the ensuing potential changes were amplified (Nihon Kohden, RP-3). Three sets of apparatus were used to simultaneously record contractions in three out of the five gonadal lobes on a multichannel pen-recorder (Nihon Kohden, RJG-3024).

Internal pressure changes were measured by placing a sea water filled glass tube, 2 mm in internal diameter, on one of the five genital pores. The space between the genital pore and the glass tube was sealed with rubber tubing. The glass tube was connected to a high-gain pressure transducer (Nihon Kohden, LPU-0.1), coupled to a pre-amplifier (Nihon Kohden, RP-3).

Repetitive electrical stimuli (10 ms, 50 Hz, 20 to 30 V) were applied for 5 to 20 seconds through a pair of silver-silver chloride electrodes (0.6 mm in tip diameter), one being placed inside the test and the other in the sea water outside the preparation. Thus the stimulation current flowed mainly through the aboral region of the test. To stimulate a gonadal lobe directly, both electrodes were placed on the surface of the lobe.

Stimulating reagents consisted of 0.5 *M* KCl and 10^{-4} *M* ACh dissolved in sea water. A few drops of these reagents were added to the preparation. To block neuromuscular transmission, the test cavity was filled with isotonic $MgCl_2$ for 15 minutes and the solution was replaced by sea water immediately before the experiments.

Results

Rhythmic contractions of the gonad

Figure 1A demonstrates the twitch-like contractions during spontaneous gamete discharge. In the absence of gamete shedding, the contraction heights were much smaller. Identical temporal coincidence was observed between the contraction pattern and the internal pressure changes of the gonad (Fig. 1B).

Electrical stimulation of the aboral region induced tetanic contractions in gonads, resulting in a vigorous gamete discharge (Fig. 2A). Those responses were always accompanied by small rhythmic contractions. Usually the gonads ceased to contract a few minutes after the stimulation. Gonads responded to ACh by an arrhythmic contraction (Fig. 2C), which became rhythmic upon electrical stimulation of the aboral region (Fig. 2D). The addition of KCl caused the gametes to shed copiously and the gonad showed tetanic contractions accompanied with rhythmic contractions (Fig. 2E).

Rhythmic and arrhythmic contractions

Arrhythmic and spatially restricted contractions were evoked when electrical stimulation was given directly to the gonadal surface. The rising and falling phases of those arrhythmic responses were very slow.

The possibility of a chemical secretion being involved in the rhythmic contractions was tested using preparations pretreated with $MgCl_2$ in order to block synaptic trans-

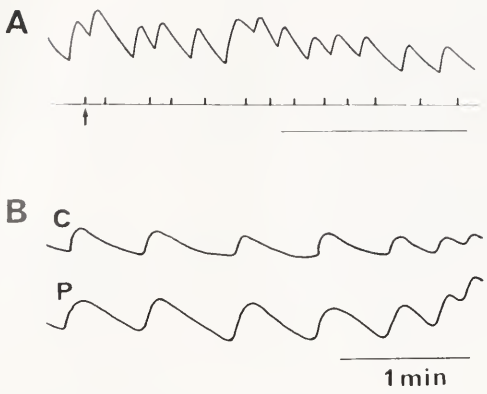

FIGURE 1. Temporal relationships among three spawning-related events. A: Rhythmic contractions of the gonadal lobe (upward deflections in the upper trace) and heavy shedding observed with the naked eye (arrow). B: Rhythmic contractions (C) and changes in the internal pressure of the lobe (P).

missions (Fig. 3). Gamete discharges were observed in response to suprathresholdal electrical stimulation. The response to the electrical stimulation of the surface of the lobe resembled that observed in the intact preparation (Fig. 3A). On the other hand,

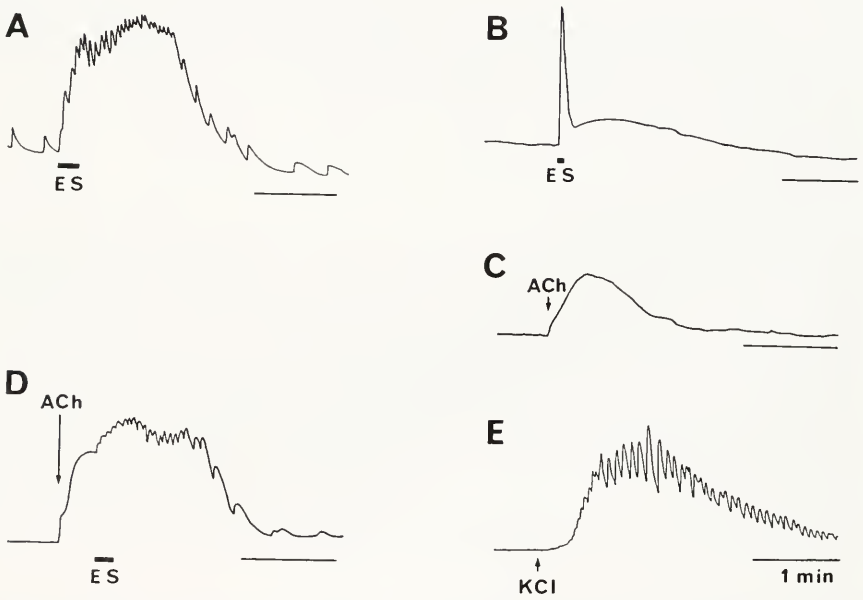

FIGURE 2. Five types of contraction pattern. A: Spontaneously and rhythmically contracting lobe responds by an incomplete tetanus-like contraction to electrical stimulation (ES) given through the aboral region of the test. Note that small rhythmic contractions persist with higher frequency. B: Lobe deprived of its own gonoduct responds to electrical stimulation (ES) to aboral region with bi-phasic contraction; initial fast twitch-like contraction is followed by an arrhythmic slow one. C: Arrhythmic contraction in response to 10^{-4} *M* ACh. D: The arrhythmic contraction induced by ACh becomes rhythmic when electrically stimulated (ES). E: Response of a quiesent lobe of the addition of a few drops of 0.5 *M* KCl into the test cavity. Note that small rhythmic contractions are superposed on the arrhythmic contraction.

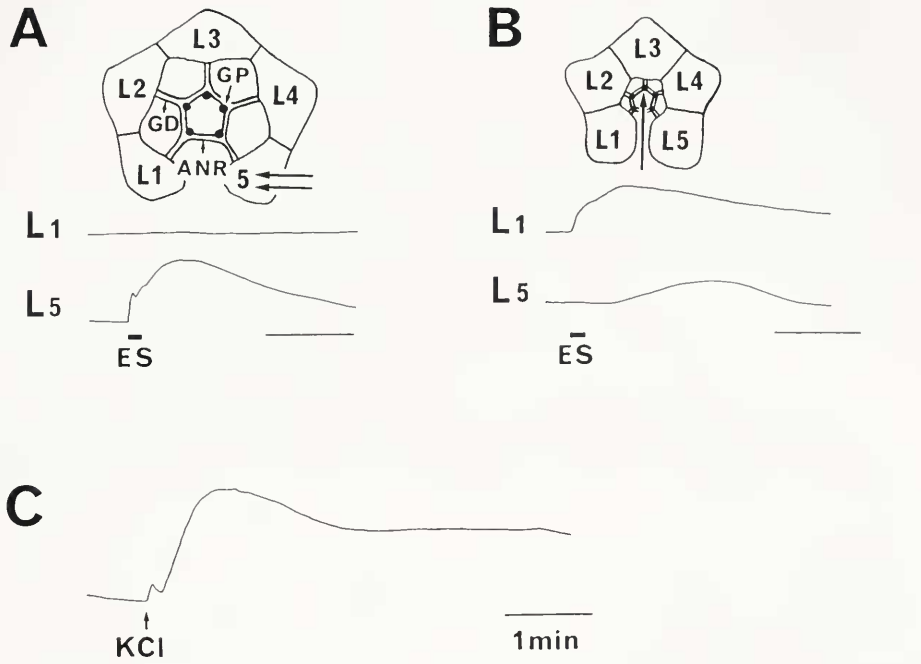

FIGURE 3. Contractions of gonad pretreated for 15 minutes with 0.5 *M* $MgCl_2$. Insets in A and B schematically show location of electrodes (thick arrows) viewed from inside. ANR: aboral nerve ring. GD: gonoduct. GP: genital pore. B: Stimulation given through the aboral region (arrow) of the test induced arrhythmic contractions in both L1 and L5 lobes. C: Arrhythmic contraction in response to addition of a few drops of 0.5 *M* KCl into the test. Rising and falling phases are slow.

responses to electrical stimulation of the aboral region (Fig. 3B) as well as to KCl application (Fig. 3C) were similar to that of the directly stimulated lobe and rhythmic movements were not initiated in any of the lobes tested.

To determine the site of the rhythm generator(s), all five lobes were first separated by cutting the lateral interconnections. Rhythmic contractions occurred normally in response to electrical stimulation of the aboral region. Separation of the lobe(s) from the test with each of the gonoduct(s) left intact did not abolish the rhythm. A preparation containing one lobe that was attached, via a gonoduct, to a small piece of the test with periproct, retained the rhythmic contractions. The rhythmicity disappeared, however, when the gonoduct was cut (L2 in Fig. 4C). Other lobes with intact gonoducts responded rhythmically (L1 and L3 in Fig. 4C).

Synchronous rhythmic contractions among gonadal lobes

The aboral nerve ring (ANR, inset of Fig. 3A) is a candidate for the rhythm generator located proximal to the gonoduct. The preparations were altered surgically as shown in insets of Figure 4. The stimulant used was KCl solution applied locally to the aboral region.

Simultaneous recordings from any three lobes showed that the phases of rhythmic contractions and the relative magnitudes were strikingly synchronized (Fig. 4A). However, supernumerary or lack of contractions occurred on rare occasions during the course of the rhythm. The synchronization was only slightly impaired by a single cut

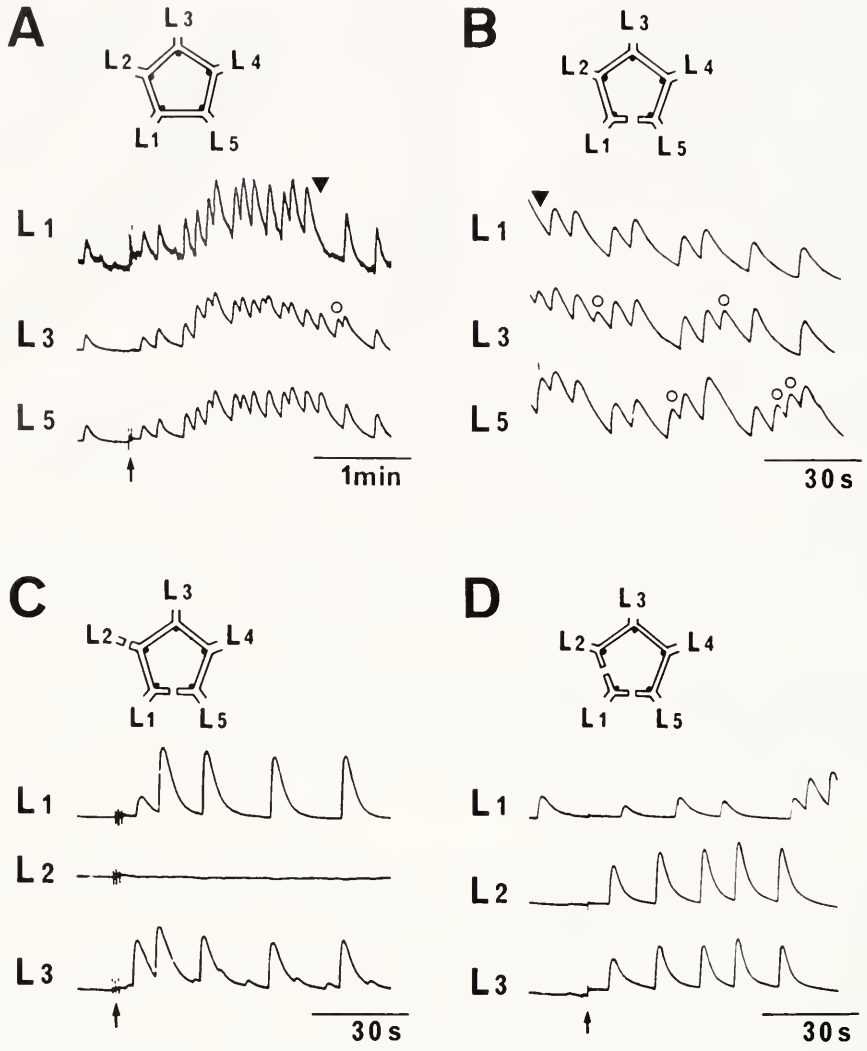

FIGURE 4. Simultaneous recordings from three lobes. A: ANR was intact. B: ANR was transected between L1 and L5. C: ANR was transected as in B with additional cut in the gonoduct of lobe L2. No contraction appeared in L2, but rhythmic contractions took place synchronously in L1 and L3. D: ANR was transected at two places, between L1 and L5 and between L1 and L2. L1 showed rhythmic contractions independently of the other two lobes. In A and B, triangles indicate lack of contraction in one lobe, while contractions took place synchronously in the other two lobes and circles indicate contractions occurring independently of the other lobes. Arrows indicate the time of KCl application.

in the ANR, although out-of-phase contractions tended to occur more frequently (Fig. 4B).

When the ANR was separated into two sectors, lobes in the same sector contracted synchronously but independently of those in the other sector (Fig. 4D). In most cases, the frequency of the rhythm was higher in the lobe connected to the larger sector.

When one out of the five gonoducts was cut transversely (Fig. 4C), the lobe (L2) with the severed gonoduct did not contract, while all other lobes contracted syn-

chronously. The spread of the rhythm is, therefore, achieved by the coordinating information passing through the ANR and reaching the lobe via the gonoduct.

Conduction of excitation through ANR

Recordings from three lobes at high speed (10 mm/s) revealed a phase difference between onsets of the earliest and latest contractions, although they appeared to be occurring synchronously in slow speed recordings. The longest delay was observed when the ANR was cut at one place (Fig. 5B). As shown in the first group of contractions, L5, which was adjacent to the cut, started to contract earliest and the delay in onset proceeded in a sequence from L3 to L1. When a lobe opposite to the cut contracted earliest as in the second group of contractions (L3), the two lobes on either side of the cut (L1 and L5) contracted with a slight delay and almost at the same time. Even when the ANR was intact (Fig. 5A), the delay in onsets of contractions was evident, although only slight (the earliest and the latest being in L1 and L3 in the first group and those, in L1 and L5 in the second group, respectively).

The conduction velocity was calculated using the longest time lag between the

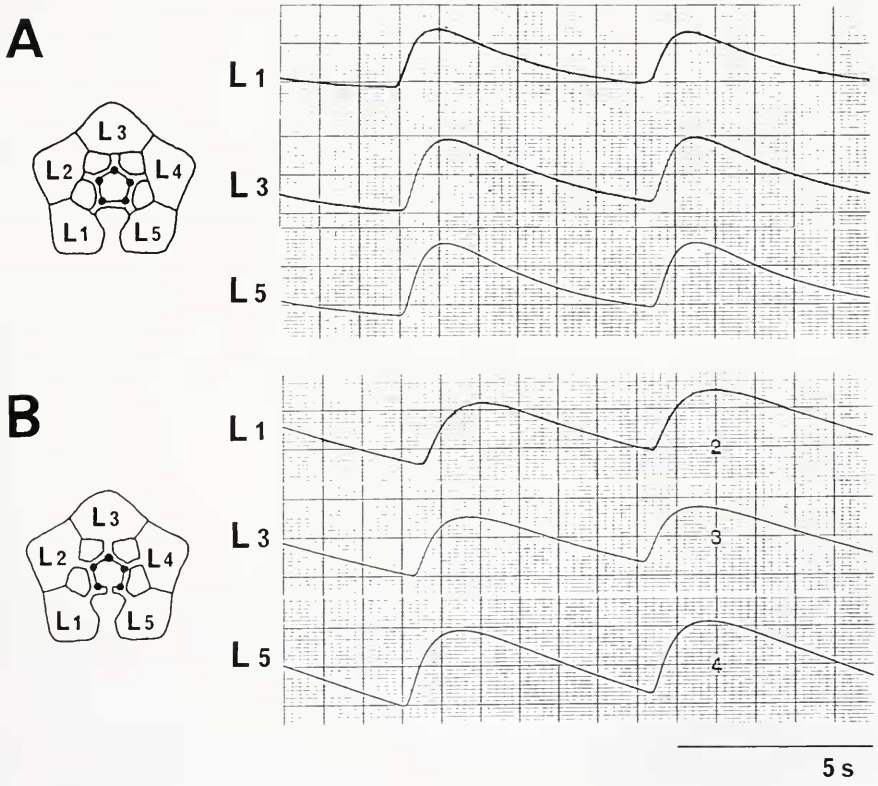

FIGURE 5. Simultaneous high speed recordings from three lobes. A and B were obtained from the same animal. In A, ANR was intact; in B it was cut as shown in the inset. A: Contractions started in sequence L1, L5, L3 at left and at right, L1, L3, L5. The time lag between onsets of the earliest and the latest contractions was 0.2 s in the left records and 0.3 s in the right. B: Contractions started in sequence at left L5, L3, L1 and at right L3, L5, L1. The time lags between onsets of the earliest and the latest contractions was 0.45 s in the left records and 0.25 s in the right.

onsets of the earliest and latest contractions and the distance between the genital pores belonging to the two contracting lobes. The time lag was 0.3 s and the distance, 9.2 mm, so that the velocity was 0.03 m/s at 28°C.

Discussion

Rhythmic contraction

The present experiments demonstrate arrhythmic and rhythmic contractions of the gonad. The arrhythmic contractions were induced by electrical stimulation of the gonadal surface or ACh application. These responses were similar to those induced upon KCL application or electrical stimulation in the $MgCl_2$ pretreated gonad, suggesting that all the stimulants act directly on the gonadal muscle. The rhythmic contractions, apparently originate from neural activity since they were abolished by a transverse cut of the gonoduct, which presumably receives input from the ANR (Chuènot, 1948 cited from Smith, 1965). In fact, nerve bundles have been observed along the wall of the gonoduct using electron microscopy (pers. comm. from Yoshida).

The arrhythmic and rhythmic types differ in the time course of contraction. Gonad with gonoduct transected responded occasionally by a bi-phasic contraction to a brief electrical stimulation (Fig. 2B); the first phase being a single twitch-like contraction and the second phase, slower and of longer duration. The first phase resembles the rhythmic contractions, indicating a common neural mechanism. The second phase was similar to the arrhythmic contraction.

The fact that both the electrical stimulation and the KCl application evoked the arrhythmic contraction (direct stimulation of gonadal muscle) as well as the rhythmic contraction (indirect effect through neural elements) requires a modification of the conclusion (Iwata and Fukase, 1964b) which assumes only the direct effect of both stimulants on gonadal muscles.

The rhythm generator appears to reside outside the gonad in the ANR; information is conducted to the gonad via the gonoduct because preparations containing one gonadal lobe attached to a small piece of the aboral test retained the rhythmic contractions. Even when the ANR was cut at both sides adjacent to the gonoduct, the rhythmicity was not abolished, suggesting that the rhythm generator is located near the genital pore where the ANR connects with the gonoduct.

Synchronization of the rhythm

The rhythmic synchronous contractions among five gonadal lobes result in simultaneous gamete shedding. Each lobe has its own rhythm generator and when generators were connected by the ANR system, contraction occurred simultaneously in multiple lobes. However, either supernumerary or lack of contraction was seen in intact preparations and even more frequently in those with ANR transected at one place (Fig. 4B), suggesting that an interacting mechanism among the five generators helps to maintain the concerted rhythmicity.

Closer examinations revealed a time lag in onsets of contractions. It is assumed that the rhythm generator, which evokes the earliest contraction, acts like a pacemaker and also excites the other generators either directly or indirectly. The results indicate that the excitation from a pacemaker is conducted in both directions, with no one generator being dominant.

For each synchronous contraction, the maximum time lag between the earliest and the last contracting lobes was smaller when the ANR was intact (250 ms) than when it was severed at one place (450 ms). This difference may be due to the difference

in spatial arrangements; the intact generators are arranged in a closed loop whereas, after severing, the arrangement is linear. Smith (1945) proposed a hypothetical model in the oral nerve ring for coordinative stepping of starfish tube feet. He assumed that each rhythm generator (motor center) innervates all the five radii. Such a scheme may not apply in the present case because the absence of contraction in the preparation with a single cut in the ANR was most frequently seen in the lobe furthest from the leading one. If the rhythm generator innervates the other four directly, then these lobes should have failed to contract. Perhaps each rhythm generator directly innervates only two adjacent generators, conducting the signals sequentially to the distal lobes.

The conduction velocity in the echinoderm nervous system has been measured by many workers: 0.04 m/s in *Echinus esculentus* (Cobb, 1968), *ca.* 0.07 m/s in *Arbacia lixula* (Millot and Okumura, 1968) and 0.14 to 0.20 m/s in *Strongylocentrotus franciscanus* (Sandeman, 1965). The conduction velocity measured by a quite different method in the present experiments gave about the same value. Our measured conduction velocity must be smaller than the true velocity for it was calculated from the delay between the earliest and the last contracted lobes, which included time required for conduction as well as the synaptic delay.

In the breeding season, the sea urchin gonads are filled with mature gametes and show rhythmic contractions of their lobes spontaneously as well as in response to artificial stimulations. When spent gonads were used, movements were only of a type of arrhythmic slow contraction. In the off season, gonads are filled with nutritive substances and gonadal lobes do not show rhythmic movements. A seasonal variation in internal conduction has been reported by Cochran and Englemann (1972) in *Strongylocentrotus purpuratus*. The concentration of spawning inducing factor in the radial nerves fluctuates annually, being highest during the breeding season. How this relates to rhythmic contraction is unknown.

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